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# Effect of Indole-3-Acetic Acid on Stress Relaxation of Japanese Black Pine Seedling*

Kazuo SUMIYA** and Tadashi YAMADA**

**Abstract**——Stress relaxation analysis was employed to determine the effect of indole acetic acid (IAA) on cell wall properties of woody plants, using Japanese black pine seedling. IAA was added on the hypocotyl in the elongating and maturing stage. The stress relaxation curve was simulated by a model of four or five MAXWELL viscoelastic components. IAA decreased the shortest relaxation time and increased the longest one in the elongating stage, but it had no effect in the maturing stage.

## Introduction

Plant cells are generally bounded by a cell wall. If a cell is to enlarge, it can do so by enlarging the cell wall. It has been considered[1,2] that the cell growth occurs as a continual series of independent extension steps, the first step of which is initiated by a biochemical modification in the extensile properties (a wall loosening) and is followed by a viscoelastic or elastic extension of the wall. If this mechanical extension step is elastic, it must be followed by a second biochemical step to render this extension irreversible. Therefore, if we are to understand how cell elongation and cell wall extension are controlled, we must know what the mechanical properties of the walls are, and how they are changed by conditions such as the presence of auxin which changes the rate of elongation.

Most recent and useful studies on the mechanical properties of cell wall have been made by using rheological techniques, such as stress relaxation and creep measurements. For example, HAUGHTON *et al.* found that the cell wall of some green algae exhibited non-linear viscoelastic behavior and apparent changes in the quasi-static modulus with temperature could be explained satisfactorily in terms of reaction rate theories for viscoelastic processes[3,4]. For auxin-induced cell elongation, stress relaxation and creep analysis were employed to determine changes in cell wall properties of *Avena* coleoptile[5,6] and green pea stem[6,7]. Their stress relaxation curves indicated that auxin reduced the relaxation modulus but did not affect the relative rate of relaxation.

In woody plants, on the other hand, it has been established that high concentrations of endogenous indolic growth substances in the cambial zone and inner phloem induced the rapid cell division in the cambial zone and differentiation of early wood tracheids[8]. Reduction of cell division and production of late wood cells were associated with reduced concentrations of endogenous indolic growth promoting substances and increased concentrations of a phenolic growth inhibitor. WARDROP[9] demonstrated that when indole acetic acid was applied asymmetrically to vertical stems of *Pinus radiata*, compression wood was formed. It was also observed that the force in the woody xylem under a constant bending restraint relaxed slowly and then recovered in growing seasons, even if the values were compensated by the diameter growth[10]. And then this force required for geotropic recovery of woody stem was partly ascribed to the formation of reaction wood, that is the abnormal distribution of lignin formation in the stem.

These facts point out the necessity to ascertain the effect of indole acetic acid on the extensibility of woody cell wall.

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## Experimentals

The seeds of Japanese black pine (*Pinus Thumbergii* PARL.) were germinated and grown on vermiculite for 4 days and 4 weeks under 20 000 lux light radiation for 14 hr/day in a chamber conditioned at 28°C and 85 % relative humidity. Hypocotyls elongated rapidly and their YOUNG's modulus increased in a week after germination but their elongation was stopped and the increasing rate of their moduli gradually decreased at about 3 weeks, as shown in Fig. 1.

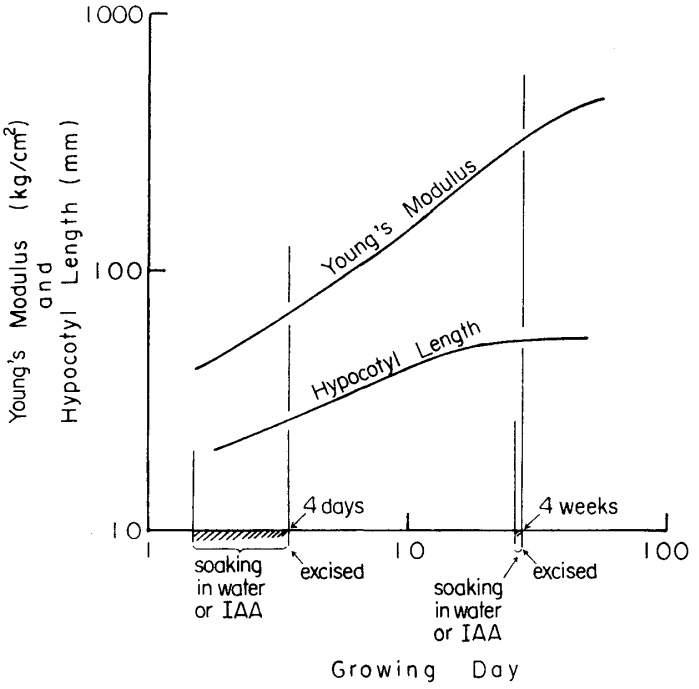


Fig. 1. Increase of hypocotyl length and YOUNG's modulus in growing days.

Hypocotyls of the seedling had been soaked in water or 10 mg/liter indole-3-acetic acid (IAA) for 2 days before a 10 mm segment was excised from the upper portion of the hypocotyl. Six of these segments, thus, prepared were immediately subjected to tensile stress relaxation test in 0.25 mole mannitol solution to examine the behaviors of the living segments which were no longer extended by turger pressure, and the other six segments were killed in boiling methanol and then tested in water to examine the behaviors of the cell wall itself.

For stress relaxation test, a Tensilon Model UTM-II tensile tester (Toyo Boldwin Co., Ltd.) was used at 20°C. For the killed segments, measurements were also made at 40° and 60°C to test time-temperature superposition. The distance between two clamps was 5 mm. The stress relaxation process was automatically recorded to give load-time curves after the segment received a constant amount of strain (2 %) which was given by lowering the bottom clamp with a speed of 20 mm/min. This constant strain was in the elastic range of stress-strain curve. The stress was calculated from the load obtained in each time and the cross sectional area of the segment, which was caluculated from its initial diameter under the supposition that the cross section was thoroughly a circle.

## Results and Discussion

Stress relaxation data of the living segments are shown in Fig. 2. In the rapidly elongated stage of hypocotyls, 10 ppm IAA solution reduced the relaxation modulus at short times as in the

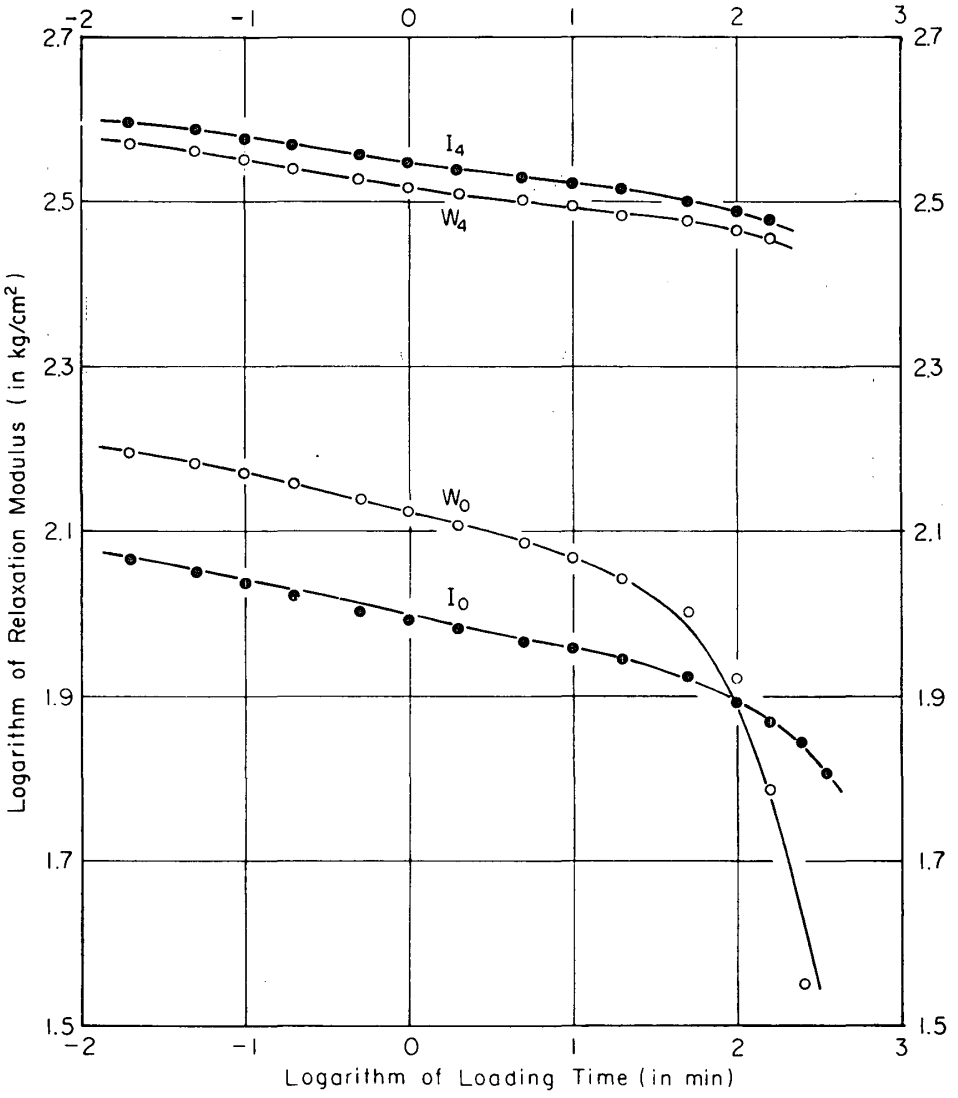


Fig. 2. Stress relaxation modulus for the living segment of pine at 20°C.
$W_0$ (open circle) : segment excised at 4 days, soaking in water for 2 days before its excision.
$I_0$ (black circle) : segment excised at 4 days, soaking in 10 ppm IAA for 2 days before its excision.
$W_4$ (open circle) : segment excised at 4 weeks, soaking in water for 2 days before its excision.
$I_4$ (black circle) : segment excised at 4 weeks, soaking in 10 ppm IAA for 2 days before its excision.
Curves were calculated by eq. (2).

*Avena* coleoptile[6], but prolonged the rapid fall of the relaxation modulus at long times that appeared in the segment which had been elongated without IAA. In the contrast, IAA which acted on hypocotyls after the stoppage of their elongation had no effect on the relaxation process of them.

Stress relaxation data of cell wall itself in each temperature are shown in Fig. 3. Attempts were then made to reduce the data to 20°C with the time-temperature superposition[11]. When each point in Fig. 3 is shifted horizontally and vertically to be superimposed and gives a continuous curve, the results are shown in Fig. 4; a single composite curve was obtained. These composite curves represent the time dependence that would have been obtained over a much wider

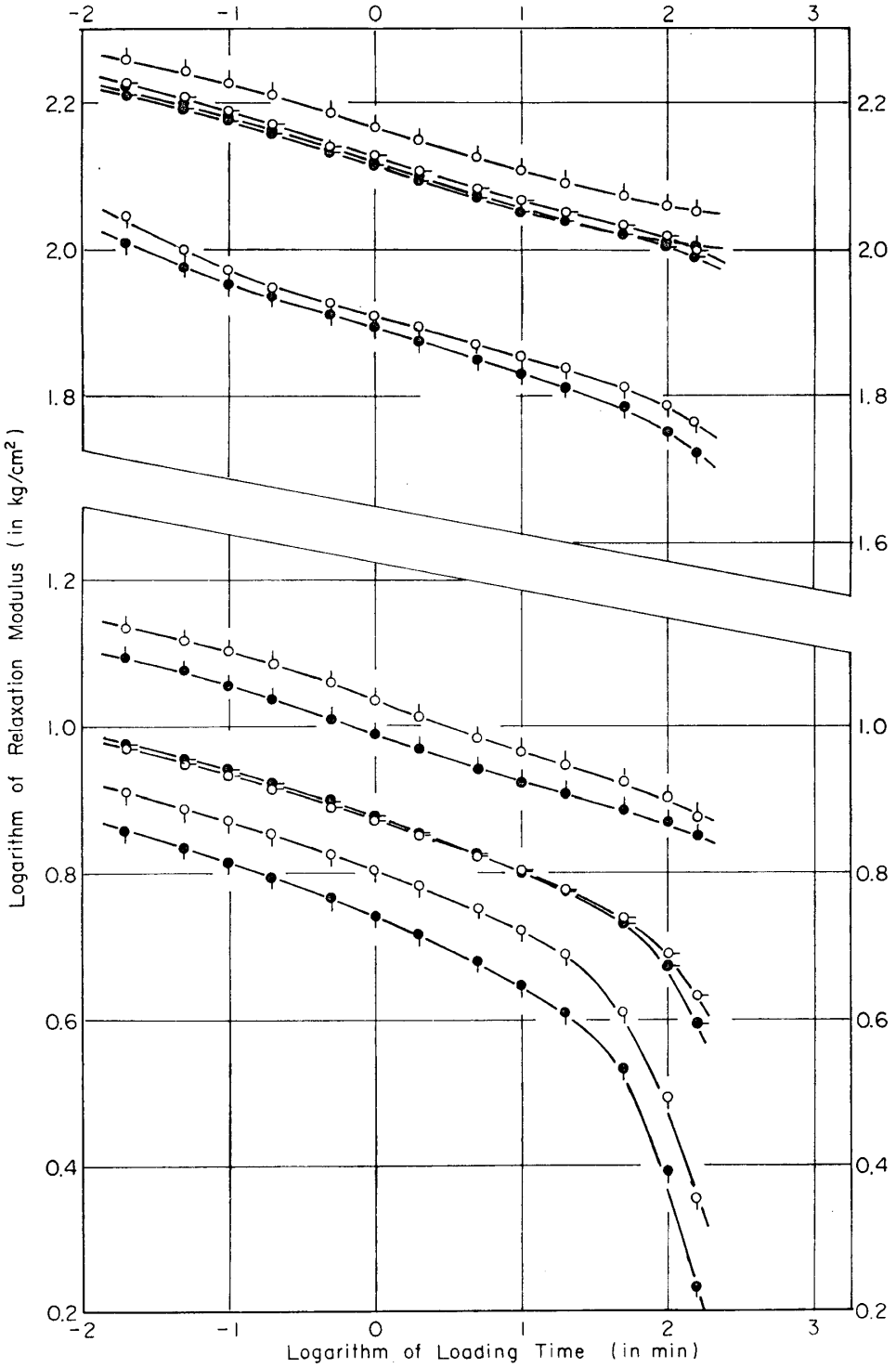


Fig. 3. Temperature dependence of stress relaxation modulus for the segment of pine killed by hot methanol.
The upper: segment excised at 4 weeks, the lower: segment excised at 4 days.
Open circle: soaking in water for 2 days, black circle: soaked in 10 ppm IAA for 2 days.
Pip up: measured at 20°C, pip right: measured at 40°C, pip down: measured at 60°C.

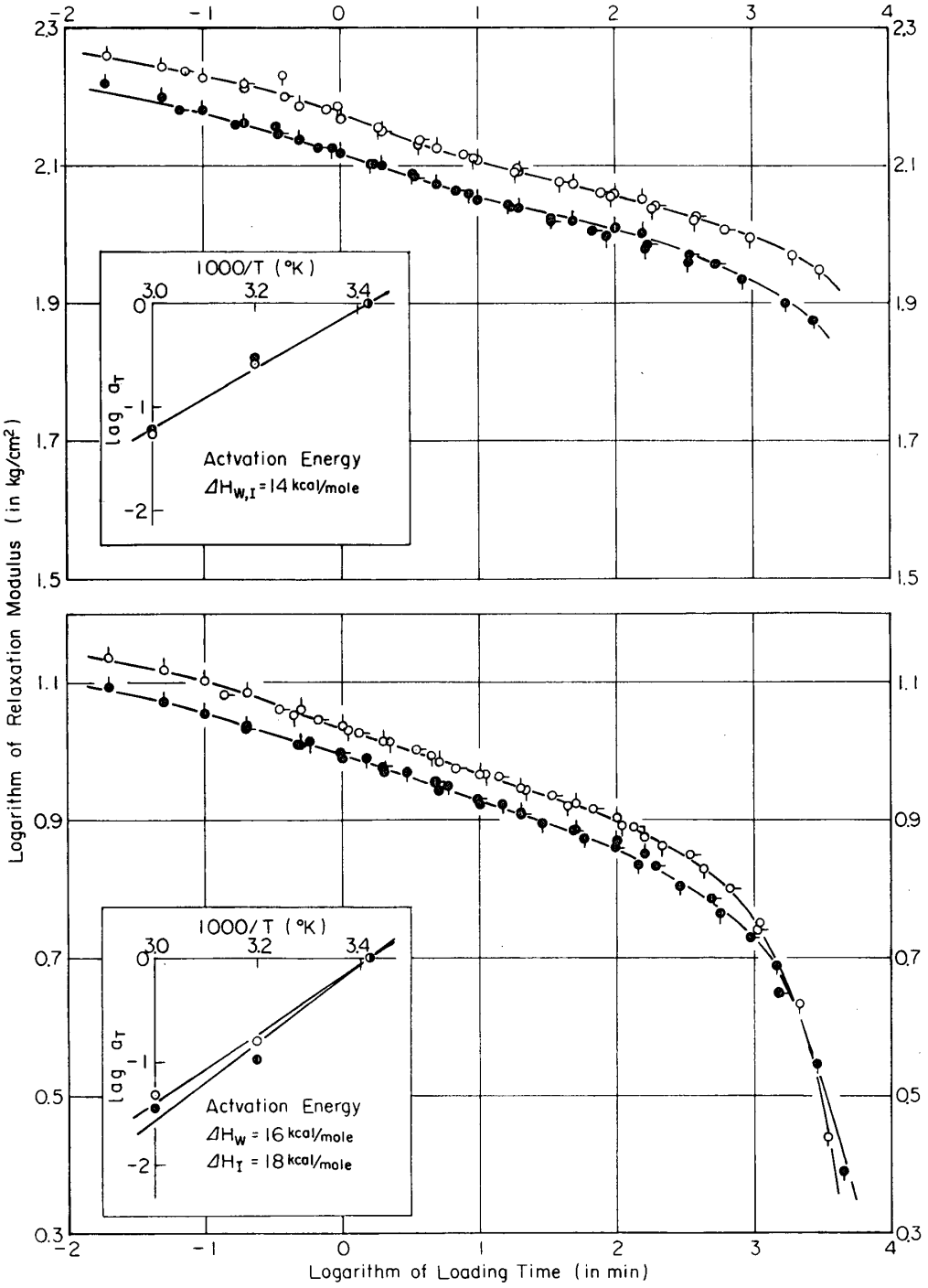


Fig. 4. Composite curves for the relaxation modulus reduced at 20°C from the data of Fig. 3, and plots of logarithm of shift factor, $a_T$, versus inverse of the absolute temperature, $T$.
The upper: segment excised at 4 weeks, the lower: segment excised at 4 days.
Open circle: soaked in water, black circle: soaked in 10 ppm IAA.
Pip up: measured at 20°C, pip right: measured at 40°C, pip down: measured at 60°C.
Curves were calculated by eq. (2).

time scale at 20°C. Here, since the values of vertical shifts fluctuated widely around the values which were expected from the time-temperature superposition theory, we did not discuss their physical meaning in more detail.

YOUNG's modulus was much lowered by the methanol treatment. Though the cause of this lowering of YOUNG's modulus is not known yet, the stress relaxation behaviors are qualitatively similar to those of the living segments, except the prolongation of the beginning of the rapid fall of stress relaxation. It can be seen that IAA acted to reduce the falling rate of relaxation modulus at long times on the cell wall in the rapidly elongated stage of hypocotyls, but had no effect on the cell wall of hypocotyls which had been stopped their elongation. These actions of IAA were similar to those on the living segments.

The apparent activation energies of these relaxation processes, $\Delta H$, were obtained from the following ARRHENIUS equation for the horizontal shift factor $\log a_T$:

$$\log a_T = \frac{1}{2.303}\frac{\Delta H}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right), \tag{1}$$

where $R$ is gas constant, $T$ is absolute temperature and the subscript 0 shows the reference temperature. So, the activation energies in these experiments, which were calculated from plotting $\log a_T$ against $1/T$ as shown in Fig. 4 also, were almost the same value. This fact seems to show that the relaxation mechanisms taken place in the cell wall of the hypocotyls which elongate rapidly with or without IAA are very similar to those in the cell wall of the hypocotyls on which IAA acts or not when their elongations cease.

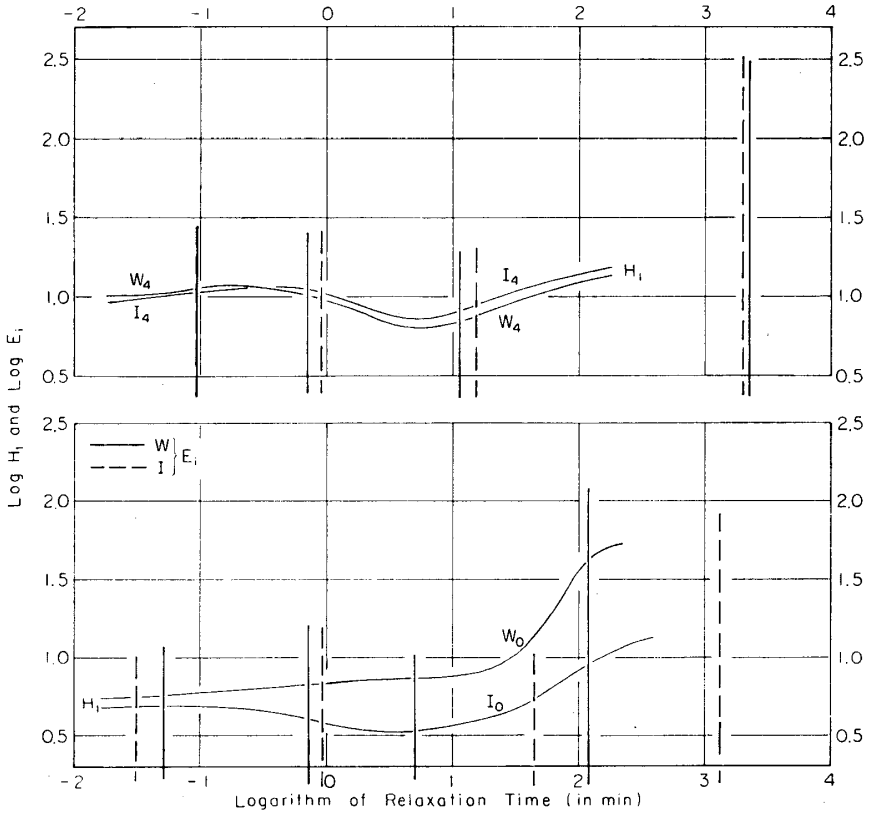


Fig. 5. Continuous ($H_1$) and discrete ($E_i$) relaxation spectra for the living segment of pine calculated by eq. (5) and Procedure X, using the data of Fig. 2.
$W_0$, $I_0$, $W_4$ and $I_4$ are identified as in Fig. 2.

Since a simple MAXWELL model is not fully applicable to the stress relaxation curves shown in Figs. 2 and 4, we should select a model, which better fits the real stress relaxation curves to analyze them in more detail. If the distribution of relaxation times is expressed as a discrete distribution, we have :

$$E_r(t)=\sum_{i=1}^{m} E_i \exp(-t/\tau_i). \tag{2}$$

A plot of $\log E_r(t)$ versus $t$ should approach a straight line for $t>\tau_m$, if a maximum relaxation time, $\tau_m$, truly exists. The slope of the line is $-1/(2.303\tau_m)$ and the intercept of the line is $\log E_m$. When the values of $\tau_m$ and $E_m$ can thus be obtained, the above equation can now be cast in the form :

$$E_r(t)-E_m \exp(-t/\tau_m)=\sum_{i=1}^{m-1} E_i \exp(-t/\tau_i). \tag{3}$$

A plot of $\log\{E_r(t)-E_m \exp(-t/\tau_m)\}$ versus $t$ should approach a straight line for $t>\tau_{m-1}$, if a discrete relaxation time truly exists, that is reasonably separated in time from $\tau_m$ and $\tau_{m-2}$. After determining $\tau_{m-1}$ and $E_{m-1}$ from the slope and intercept, the process can be repeated to find $\tau_{m-2}$, $E_{m-2}$, etc. This procedure was proposed and called Procedure X by TOBOLSKY and MURAKAMI[12].

By using this procedure, we got a model consisting of at least four MAXWELL components for

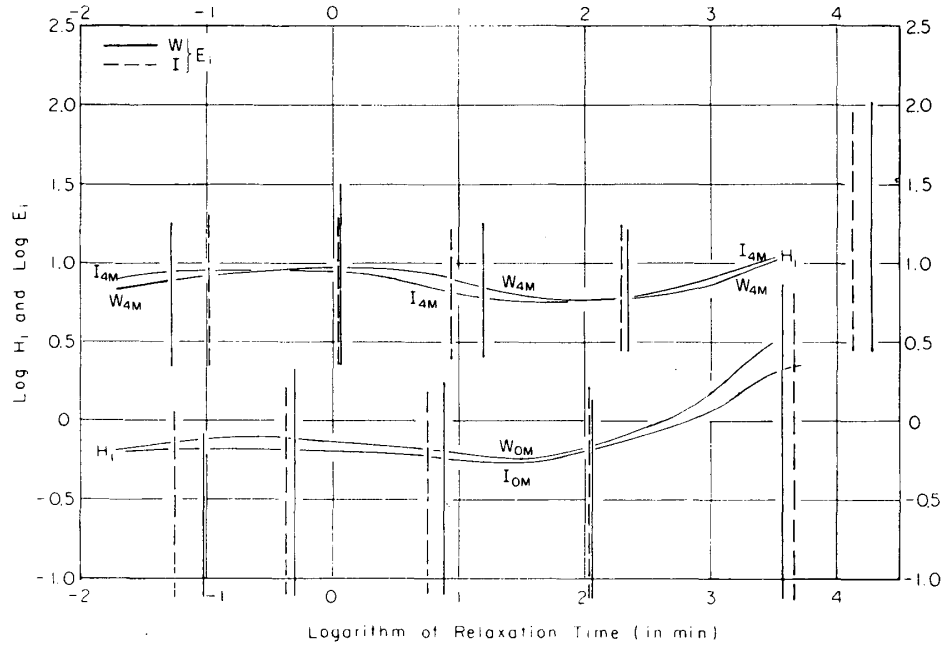


Fig. 6. Continuous ($H_1$) and discrete ($E_i$) relaxation spectra for the killed segment of pine calculated by eq. (5) and Procedure X, using the data of Fig. 4.

$W_{0M}$ : segment excised at 4 days, soaking in water for 2 days before its excision, and then killed by hot methanol.

$I_{0M}$ : segment excised at 4 days, soaking in 10 ppm IAA for 2 days before its excision, and then killed by hot methanol.

$W_{4M}$ : segment excised at 4 weeks, soaking in water for 2 days before its excision, and then killed by hot methanol.

$I_{4M}$ : segment excised at 4 weeks, soaking in 10 ppm IAA for 2 days before its excision, and then killed by hot methanol.

the living segment and a model consisting of at least five ones for the cell wall itself. The YOUNG's modulus, $E_i$, and relaxation times, $\tau_i$, of each of these MAXWELL components are shown as vertical lines in Figs. 5 and 6 for the living and killed segments respectively. The curves shown in Figs. 2 and 4 are stress relaxation curves, $E_r(t)$, calculated by eq. (2) using each of these $E_i$ and $\tau_i$.

In experimental analysis of stress relaxation data, the continuous distribution function $H(\tau)$ that expresses the distribution of relaxation times is widely used :

$$E_r(t) = \int H(\tau) \exp(-t/\tau) d \ln \tau. \quad (4)$$

Approximation methods have then been devised to obtain $H(\tau)$ curves from the experimental $E_r(t)$ curves by graphical procedures. The first approximation method of ALFREY gives :

$$H_1(\tau) = -(1/2.303)[dE_r(t)/d \log t]_{t=\tau}. \quad (5)$$

The approximated distribution curves of relaxation times in these experiments, $H_1$, are also shown in Figs. 5 and 6. Comparing the discrete distribution of relaxation times with this continuous one, it is confirmed that the former is sufficient to analyze the stress relaxation curve.

From Figs. 5 and 6, it may be assumed that when 10 ppm concentration of IAA is applied on the rapidly elongated hypocotyl, it changes the relaxation process at the longest times, which mainly contributes to YOUNG's modulus of the segment, to the process which is more difficult to relax. The mechanisms of this process have not been clarified at all. But it is conceivable that the process is partly concerned with the maturation of cell wall, because the stress relaxation behavior of the living segment applied IAA in the rapid elomgation stage becomes to be resemble to the one in the more matured stage, as shown in Figs. 2 and 5.

## References


1) R. CLELAND, Ann. Rev. Plant Physiol., **22**, 197 (1971).
2) R. CLELAND, Plant Physiol., **47**, 805 (1971).
3) P. M. HAUGHTON, D. B. SELLEN and R. D. PRESTON, J. Exp. Botany, **19**, 1 (1968).
4) P. M. HAUGHTON and D. B. SELLEN, ibid., **20**, 516 (1969).
5) R. CLELAND, Planta, **74**, 197 (1967).
6) R. YAMAMOTO, K. SHINOZAKI and Y. MASUDA, Plant Cell Physiol., **11**, 947 (1970).
7) E. TAMIMOTO and Y. MASUDA, ibid., **12**, 663 (1971).
8) T. T. KOZLOWSKI, Growth and Development of Trees, Vol. II, 104, Academic Press (1971).
9) A. B. WARDROP, Cellular Ultrastructure of Woody Plants (ed. by W. A. CÔTÉ), 371, Syracuse Univ. Press (1965).
10) Y. HASEGAWA, T. YAMADA and K. SUMIYA, Wood Research, No. 49, 1 (1970) (in Japanese).
11) J. D. FERRY, Viscoelastic Properties of Polymer, 2nd ed., 292, Wiley (1970).
12) A. V. TOBOLSKY and K. MURAKAMI, J. Polym. Sci., **40**, 443 (1959).